IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Le Febre, David
SERIAL NO.:	09/526,920
FILED:	03/16/00
CONFIM. NO.:	1445
FOR:	ELECTROMOBILITY FOCUSING CONTROLLED CHANNEL ELECTROPHORESIS SYSTEM
ART UNIT:	1741
EXAMINER:	J. Starsiak
DOCKET NO.:	T8526

CERTIFICATE OF DELIVERY

I hereby certify that this correspondence is being sent via facsimile to the Commissioner for Patents, Attention J. Starsiak, facsimile no. 703 872-9756 on the date indicated below.

Judy Anderson

March 7, 2003
Date of Deposit

LETTER

Assistant Commissioner of Patents
Washington, D.C. 20231

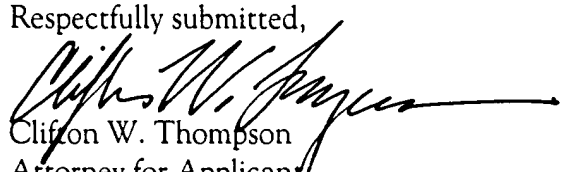
Sir:

Please find enclosed requested material, which may be helpful in examination of the case. Per the request of Examiner Starsiak, empirical data and test results from early devices in accordance with the disclosure is set forth in the attachments to this letter (6 pages). The results demonstrate that a field intensity gradient with respect to position in the channel can be created, and that analyte species can be separated. Later development work has refined and improved the technique and results, but these early devices

demonstrate that separation by means of a electric field intensity gradient and an opposing flow-induced counter force can be done in accordance with the specification.

DATED this 7th day of March , 2003.

Respectfully submitted,



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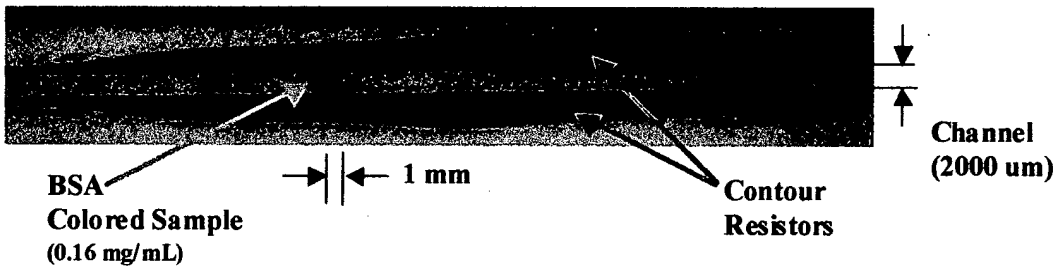
Experimental Results

(Extracted from a NIST Proposal, prepared June 2002)

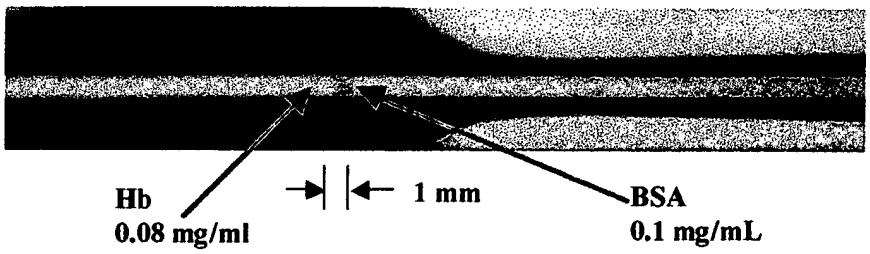
Several experiments to demonstrate EMF were performed in the year 2001 with mixed results. The top portion of Figure 5 shows a dyed blue BSA (bovine serum albumin) protein being focused within the channel at approximately 110 mm from the high voltage supply. The bottom portion of Figure 5 shows a dyed blue BSA protein and a dyed brown Hb protein being focused at 63 mm from the high voltage supply. The observed behavior of the proteins within the channel confirms that an electric field intensity profile does indeed function as a gradient force against a counter force of flow. In other words the basic concept works:

1. Proteins are focused within the channel
2. The counter force, flow, moves the proteins within the channel in relationship to the charge on the protein and possibility protein cross section
3. The electric field intensity "appears" to be linear, i.e. no obvious anomalies
4. The gradient and counterforce seem to prevent wall adhesion

However, the separation process only lasts about 15 or 20 minutes. After this period of time hydrolysis effects make the protein locations unstable. Adding chemicals that react with the hydrolysis gases or decreasing the applied voltage increases the separation time. Other techniques for extending the process time are being investigated.



Conditions: Flow rate 1.5 uL/min, 1500 volts, Linear profile



Conditions: Flow rate 1.5 uL, 500 volts, Linear profile (software enhanced)

Figure 5. Experimental Results

Multiple Gradient Segment Assembly

(The experimental data was taken Sept. 23, 2001)

The Electric Field Intensity Segments

This is a design exercise to study the ability to generate multiple electric field intensity gradient segments in a single assembly. A series of four random electric field intensity segments are generated as indicated below: (*The first numbers in the segment description are the channel locations that the electric field intensity is valid for, followed by the electric field intensity and the change in electric field intensity per interval.)*

1) 0-50 mm, E(x) = -50 to –40 V/mm in increments of 0.2 V/mm
2) 51-100 mm, E(x) = -40 to –3.25 V/mm in increments of 0.75V/mm
3) 101-150 mm, E(x) = -3.25 to –0.205 V/mm in increments of 0.0459V/mm
4) 151-180 mm, E(x) = 0.205 to –0V/mm in increments of 0.00683 V/mm

The channel is 800μm x 50 μm x 177 mm (2 mm to 179 mm) with a central channel resistor of 100 kilohm/mm (1000 μm x 180 mm) resulting in a total combined resistance of 3,408,201.55 ohms. The applied voltage has been selected at 3,408.2 volts to produce a 1 ma current through the assembly (3.4 watt power dissipation). The electrolyte is assumed to be at least a factor of 10 greater than the central resistor – the objective is to have most of the current flowing in the central resistor and not the electrolytic channel. The measurement is made with the cover plate off and with or without a high impedance electrolyte. The probe contacts the central resistor below the electrolyte. Page 4 shows the top view of the assembly and the shape and value of the contour resistors.

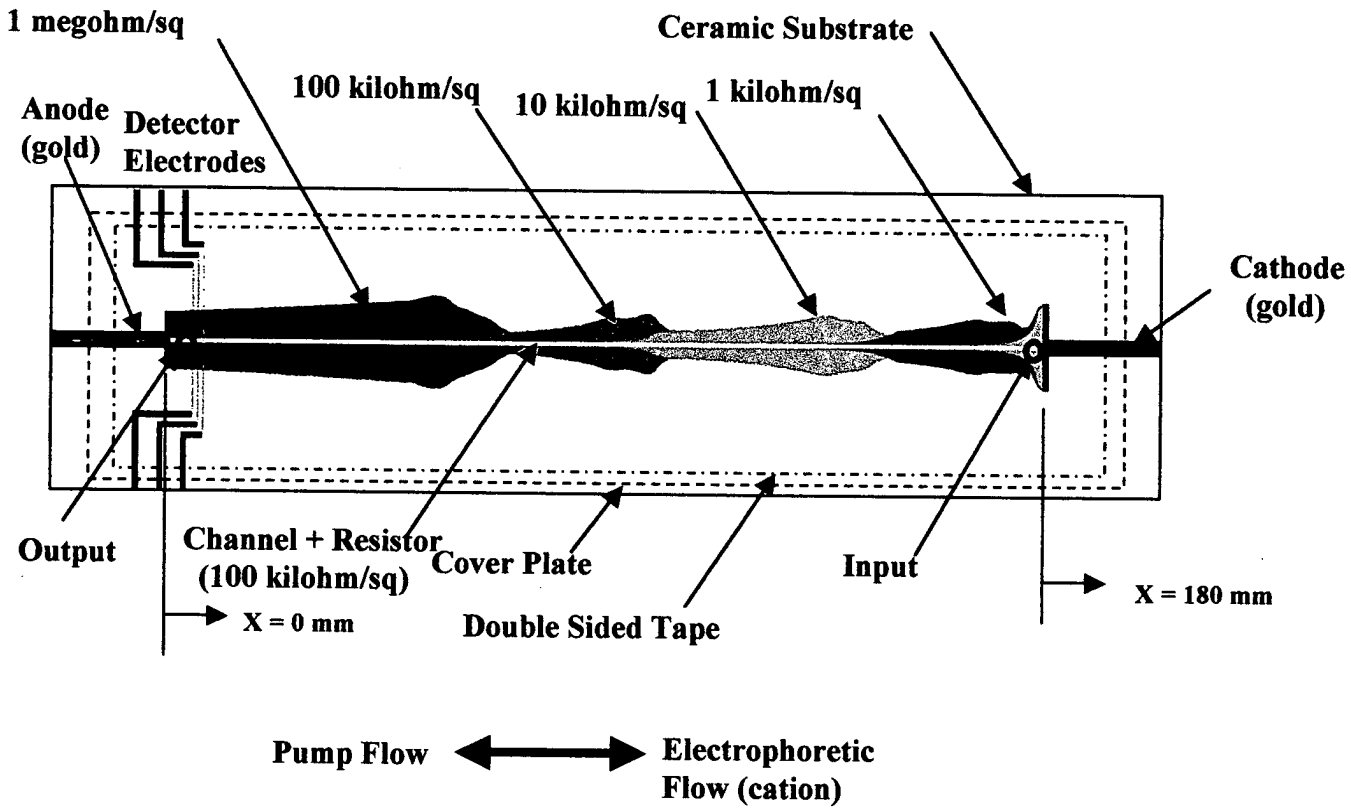
Measurement: The voltage measurements are made with a Fluke87 III digital voltmeter, which has its highest resolution under 5 volts DC. Therefore all measurements use a precision 5 volt DC reference across the channel. The substrate is placed in an x/y mill table with digital readouts. The readouts are accurate to +/- 5 microns and repeatable to +/- 3 microns. A 1 mm diameter tool steel drill bit is used to probe the channel. The drill is attached to a voltage follower (Burr Brown OPA 121) with a 1 second time constant. Each measurement is made after a dwell time of 1 minute. Note: points 55 and 60 are anomalous and caused by the shape of the piecemeal approximation. The table on page 3 records the experimental measurements, which are scaled to the design voltage and finally the electric field intensity is calculated. The measured data points and the defined data points are plotted on page 5.

Substrate. Distributed resistors of 1 megohm/sq, 100 kilohm/sq, 10 kilohm/sq, 1 kilohms/sq and 100 ohm/sq are used to create the electric field intensity profile. See Multiple Gradient Segment Assembly for resistor shapes (page 4). The resistors are made of Ruthenium Lead Oxide and are approximately 10 microns thick. The electrodes are gold and the substrate is 99% alumina (0.25 inches thick). Page 6 is a photograph of the measured substrate.

Measured Channel Voltage
for the
Multiple Gradient Segment Assembly

The Channel is measured using a precise 5 volt reference at the anode (cathode ground) and then scaled to 3408.2 volts for a 1 ma quiescent current through the channel network. The difference in scaled voltage between measurements is then divided by 5 to convert the results to volts/mm. ***(The voltage reference is 5.000 +/- 0.0005 volts and the positional accuracy of the probe is +/-5 microns. The probe has an input impedance of*** $>10^{14}$ **ohms).**

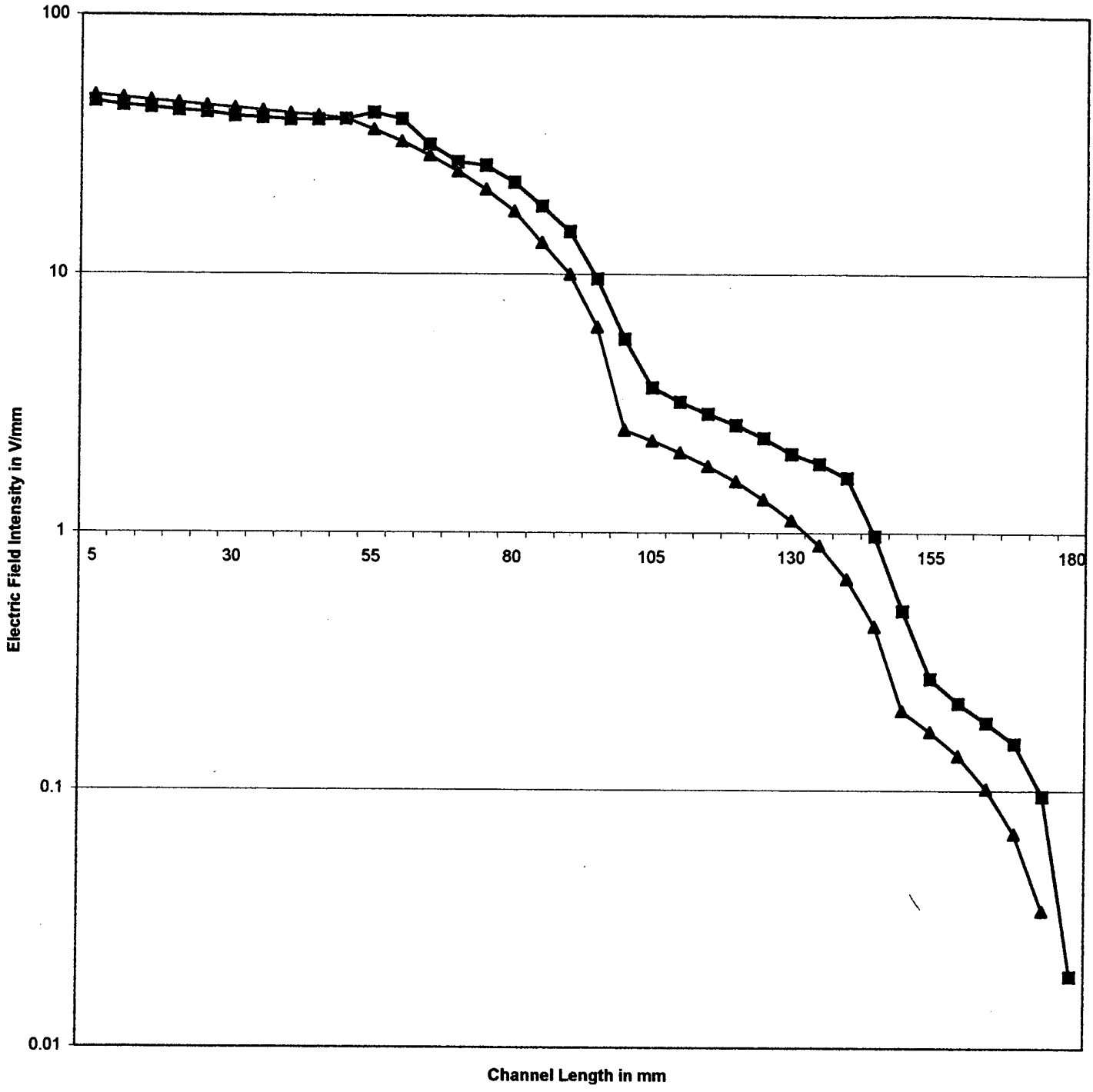
Length in mm	Measured voltage using 5 Volt ref.	Scaled to operating voltage: 3408.2 V $(681.64)(V_{measured})$	Calculated E(x) in V/mm $[(V_1 - V_2)/5]abs$
0	5	3408.2	
5	4.658	3175.07912	46.624176
10	4.328	2950.13792	44.98824
15	4.004	2729.28656	44.170272
20	3.688	2513.88832	43.079648
25	3.377	2301.89828	42.398008
30	3.077	2097.40628	40.8984
35	2.782	1896.32248	40.21676
40	2.492	1698.64688	39.53512
45	2.202	1500.97128	39.53512
50	1.9099	1301.864236	39.8214088
55	1.601	1091.30564	42.1117192
60	1.3093	892.471252	39.7668776
65	1.0764	733.717296	31.7507912
70	0.8774	598.070936	27.129272
75	0.6846	466.650744	26.2840384
80	0.5188	353.634832	22.6031824
85	0.3844	262.022416	18.3224832
90	0.2772	188.950608	14.6143616
95	0.2068	140.963152	9.5974912
100	0.1656	112.879584	5.6167136
105	0.1389	94.679796	3.6399576
110	0.1154	78.661256	3.203708
115	0.09426	64.2513864	2.88197392
120	0.07512	51.2047968	2.60931792
125	0.05802	39.5487528	2.3312088
130	0.04321	29.4536644	2.01901768
135	0.02967	20.2242588	1.84588112
140	0.01771	12.0718444	1.63048288
145	0.01058	7.2117512	0.97201864
150	0.00692	4.7169488	0.49896048
155	0.00492	3.3536688	0.272656
160	0.00331	2.2562284	0.21948808
165	0.00196	1.3360144	0.1840428
170	0.00084	0.5725776	0.15268736
175	0.00014	0.0954296	0.0954296
180	0	0	0.01908592

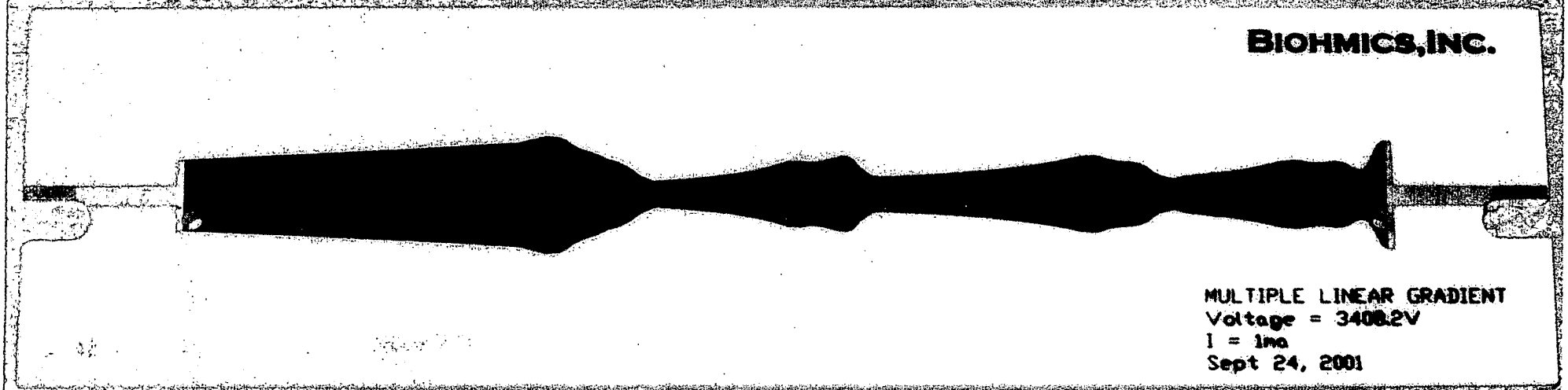


Multiple Gradient Segment Assembly

David A. Le Febre

Electric Field Intensity Pl ts
Measured (sq.) and Defined (tri.)





Substrate without coverplate

03/02/2003